RESEARCH ARTICLE

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Effects of different industrial cannabis (*Cannabis sativa* (Linnaeus 1753) (Cannabaceae)) genotype extracts on *Diuraphis noxia* Kurdjumov, 1913 *Myzus persicae* Sulzer, 1776 and *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae)

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Abstract

In this study, the effect of methanol extracts of three different genotypes (Narlısaray, Kavacık, Maltepe) of *Cannabis sativa* L. on *Diuraphis noxia* Kurdjumov, *Myzus persicae* (Sulzer) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) were investigated. In the first stage of the study, 10% concentrations of each cannabis extract were applied on the 2nd and 3rd nymphal stages of aphid species by spraying method. After the end of 24 - 48 and 72 hours of the applications, the alive and dead individuals were recorded and mortality rates were determined. In the second stage, the genotype with the highest effect was used in dose-death trials and LD_{50} and LD_{90} values at different doses (2.5%, 5%, 7.5% and 10%) were specified. In the census after 72 hours, Narlısaray genotype showed the highest mortality rate with 54.04% on *D. noxia*. While the effect of Kavacık genotype on *M. persicae* was found as 23.13%, the highest toxicity record of the same genotype was determined on *A. fabae* (as 91.76%). According to the dose measurement studies of Kavacık genotype on *A. fabae*, LD_{50} and LD_{90} values were calculated to be 0.33 and 0.110 (mg/individual), respectively. At the results of study, it has been observed that extracts of different genotypes of the industrial cannabis plant are found effective on aphid species and it is thought that they can be used in controlling of these pests.

Keywords: Aphids, Bio-pesticide, Biological control, *Cannabis sativa*, Plant extract

INTRODUCTION

Recently, many different plant-based products (botanical pesticides) are cultivated intensively in agricultural areas to control pest insects which affect negatively yield and yield parameters. Aphids (Hemiptera: Aphididae) are one of the most important harmful insects that cause damage to various cultivated plants (such as cereals, fruit trees, vegetables, etc.) and restrict crop yields (Baumann et al., 1995). Approximately, 5000 species belonging to 493 genera and 24 subfamilies of aphids are well-known in the world (Favret, 2019; Baki et al., 2020). So far, nearly 570 aphid species have been identified in Turkey (Görür, 2020). Aphids cause significant damage to plants by being a vector for plant virus diseases as well as causing direct sucking damage to the plant. Aphids cause fumagine by promoting the development of saprophytic fungi due to the honeyed substance they secrete. (Von Dohlen et al., 2006; Stevens and Lacomme, 2017; Helvacıoğlu and Akşit, 2020; Satar, 2020).

As with many plant pests, the most commonly used method of controlling aphids is also chemical control. Therefore, with the intensive usage of synthetic insecticides, aphids have developed stronger resistance to many insecticides over time (Elbert et al., 2008). As a result of excessive use of insecticides, the

environment and human health are adversely affected, the natural balance is disturbed, and residue problems occur on the plant parts (Grdıša and Gršić, 2013; Gill and Garg, 2014, Rother, 2018). Due to these and similar effects, alternative natural plant-derived compounds have been sought, which have shorter degradation times, are effective only on the target organisms, and have little negative impact on the environment (Arnason et al., 1989; Feng and Isman, 1995; Wewetzer, 1995; Hedin et al., 1997; Momen et al., 1997; Liao et al., 2017; Kunbhar et al., 2018). Plant-based extracts and essential oils attract attention as a good alternative to chemicals due to the range of bioactive chemicals they contain against plant pests (Isman, 2000; Kim et al., 2003b; Govindarajan et al., 2016; Khan et al., 2017; Sammour et al., 2018). Many of the volatile compounds found in plants are rapidly decomposed in nature and do not accumulate in the environment like other chemicals, so they could be preferred in biological control (Arnason et al., 1989; Hedin et al., 1997; Regnault-Roger et al., 2012). These compounds pose a low risk to non-target organisms, i.e., predators and parasitoids, and they are mostly non-toxic to mammals (Scott et al., 2003). Today, many researchers reveal the insecticidal activities of essential oils and their chemical components. (Regnault-Roger et al., 1993; Regnault-Roger and Hamraoui, 1995; Golob et al., 1999; Weaver and Subramanyam, 2000; Kéita et al., 2001; Lee et al., 2001; Papachristos and Stamopoulos, 2002; Kim et al., 2003a; Isman and Miresmailli, 2011; Miresmailli and Isman, 2014; Regnault-Roger et al., 2012; Pavela and Benelli, 2016; Chaubey, 2019; Feng et al., 2020; Gaur and Kumar, 2020; Sayed et al., 2021). Herbal extracts or oils obtained from plants have different advantages over pests when compared to chemical insecticides. Secondary metabolites derived from some plant species act on physiological or behavioral adaptations in the target organism and they contain many components with mechanisms that slow down the evolution of insects in these parts. When their side effects (toxicities) are evaluated for mammalians, very few compounds were found to be toxic to mammalians (Isman, 2006). Cannabis ((*Cannabis sativa*) *Linnaeus* 1753) *(Cannabaceae)*) is one of the plants that attract the attention of researchers being as a potential botanical insecticide due to the terpenoids (limonene, linalool and pinene) and phenolic compounds in it (McPartland and Sheikh, 2018).

In this study, the toxicity of the extract obtained from different genotypes of *C. sativa* against *Diuraphis noxia* (Kurdjumov, 1913), *Myzus persicae* (Sulzer, 1776) and *Aphis fabae* (Scopoli, 1763) (Hemiptera: Aphididae) was evluated, aiming their usage in integrated control of aphids on arable crop plants.

MATERIALS AND METHODS

Producing of the plant extracts

The leaves of Narlısaray genotype were obtained from Yozgat Bozok University Boğazlıyan Vocational School Agricultural Experimental Area, and the local Kavacık and Maltepe genotypes were from the campus trial areas in 2020. The leaves of the cannabis plant were dried in room conditions without direct sunlight and were ground with the help of a grinder and stored in dark conditions. A hundred g of ground plant samples were weighed and 500 ml of methanol was added to it, and it was kept in an Erlenmeyer flask for 24 hours. The plant suspensions were filtered through filter paper to separate the plant parts. Herbal extracts were obtained by evaporating the solvent in the obtained suspension with an evaporator (Buchi R-3). The obtained extracts were stored in the refrigerator at + 4°C (Alkan and Gökçe, 2012).

Plants rearing

In order to feed different aphid species, different plant species were grown for each aphid. Broad bean (*Vicia faba* (Fabaceae)), pepper (*Capsicum* sp. (Solanaceae)) and wheat (*Triticum* sp. (Poaceae)) plants used in the experiments were grown in the plastic containers (200 ml) containing soil: peat in a 1:1 ratio. Production was carried out in the climate room of Yozgat Bozok University, Faculty of Agriculture, at 25±1 °C and 60±5% relative humidity and under 16:8 (light: dark) lighting conditions.

Aphids rearing

Individuals of the last stage of nymph and/or adult D. noxia, M. persicae, and A. fabae were transferred to broad beans, pepper, and wheat plants when they reached a sufficient height (15 cm) and leaf number (over 6 pieces) to be used in the experiments, and they were reared in separate environments. The initial population of aphid cultures transmitted to the clean plants was obtained from the mass production at Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection. The reared aphids were placed into the cages (50x50x50 cm) covered with tulle in order to prevent the mixing of individuals. To ensure the continuity of mass production, aged and decaying plants were replaced with clean ones at weekly intervals. Rearing of aphid species was performed in a climate room with 25±1 °C, 60±5% relative humidity, and 16:8 (light: dark) lighting conditions.

Toxicity tests

Single Dose Death Trials

In the experiments, 2nd and 3rd instar aphid nymphs were used. The aphids that were selected to carry out contact toxicity studies were transferred to petri dishes with a 90 mm in diameter. 10% (w/v) concentrations of the extracts were used in single-dose death trials, and 10% (w/v) concentrations were sprayed onto each petri dish with a 20 ml small handheld sprayer (Erdoğan and Yıldırım, 2013). For the control group, 50% acetone/water was sprayed with the same method. The treated individuals were transferred to petri dishes

containing clean plant leaves with the help of a sable brush. At the end of the application, petri dishes were incubated at 26±1 °C, 65±5% relative humidity, and 16 hours of illumination. Experiments were set up with 10 replications, and 10 individuals were used in each replication. Mortality rates of the aphid individuals were recorded after 24, 48 and 72 hours.

Dose - Death Trials

Contact effect dose-death trials were conducted with plant extracts whose effect was determined as a result of single dose death trials. For this purpose, LD_{50} and LD_{90} values were determined by applying the genotype extracts with promising results on pests at different doses (2.5%, 5%, 7.5%, and 10%). Experiments were set up with 10 replications, and 10 individuals were used in each replication. Mortality rates in individuals were recorded after 24 and 48 hours.

Statistical analysis

The single-dose contact data were calculated as a percentage and then normalized using arcsin transformation. The data then were subjected to variance analysis (ANOVA) (P≤0.05) and the Tukey test ((P≤0.05) for differentiating treatments using the SPSS® 20 statistical software program. Probit analysis was used to calculate LD_{50}, and LD_{90} values and 95% confidence intervals for dose-response contact bioassay data using SPSS® 20 statistical software.

RESULTS AND DISCUSSION

Single Dose Death Results

In the study, the insecticidal activity of methanol extract obtained from 3 different genotypes of cannabis plant on *D. noxia*, *M. persicae* and *A. fabae* was tested. The Narlsaray genotype was found to be more effective against D. noxia than the other two cannabis genotypes at all times. While Kavacık (21.03%) genotype showed the lowest efficiency on *D. noxia*, Narlısaray genotype caused 62.32% mortality after 72 hours (F=26.23; df= 3.36; P<0.05). (Table 1).

Table 1. Toxicity of cannabis extracts on *Diuraphis noxia*

	Mortality rates (%)±SEM		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	3.03±0.35 b	5.62±0.63 b	5.62±0.63 c
Maltepe	8.73±0.29 b	17.38±0.10 b	25.32±0.15 b
Narlısaray	35.58±0.17 a	49.89±0.29 a	62.32±0.16 a
Kavacık	2.90±0.50 b	13.07±0.59 b	21.03±0.56 b

*Means in a column followed by a different lowercase letter represents results are significantly different according to Tukey test (p<0.05)

In studies conducted with *M. persicae*, the Kavacık genotype was found to be more effective than the other two genotypes at all times. After 72 hours, the Kavacık genotype caused 27.27% mortality on *M. persicae* nymphs (F=4.02; df= 3.36; P<0.05) (Table 2).

The Kavack genotype had the highest toxicity on Aphis fabae and M. persicae in the different genotype extracts of cannabis. At the end of 72 hours, Kavacık genotype caused 96.55% mortality on *A. fabae* nymphs. On the other hand Maltepe genotype showed the lowest efficiency with 71.39% mortality (F=64.79; df=3.36; P<0.05) (Table 3).

Dose Mortality Results

As a result of the single-dose screening test, Kavacık genotype showed the highest toxicity amongst all *C. sativa* genotypes against *A. fabae*. According to the dose measurement results of the Kavacık genotype, the LD_{50} value was calculated as 0.40 mg/individual after 24 hours, while the LD_{90} value was found to be 0.159 mg/individual. At the 48th hour of the study, the LD_{50} value was calculated as 0.33 mg/individual, while the LD_{90} value was determined as 0.110 mg/individual (Table 4).

Table 4. Results of dose-death trial of *Cannabis sativa*-Kavacık genotype on *Aphis fabae*

DISCUSSION

As a result of screening the insecticidal activity of different cannabis genotypes on the tested insects, the Narlısaray genotype caused the highest mortality rate on *D. noxia* nymphs (64.32%), while the Kavacık genotype had the highest mortality rate on *M. persicae* (27.27%) and *A. fabae* (96.55%). The study showed that the numbers of dead individuals were recorded after 24 and 72 hours, and the mortality percentage increased as the exposure time to the extract increased in all aphid species, depending on the time. Ahmed et al. (2020) found the efficacy of *Artemisia argyi* (L.) extract against *Brevicoryne brassicae* L. and while the LC_{50} value was found to be 38.6 mg/mL at the 24th hour, it was 3.91 mg/mL at the end of 72th hour in the study. Yadav and Patel (2018) concluded that the efficacy of *Cassia angustifolia* on *M. persicae* was 46.67% at the end of the 24th hour, while the mortality rate increased to 93.33% at the end of the 72nd hour.

The current study's findings suggest that the contents of all cannabis genotype extracts have different toxic effects on the aphid species tested. Peña-Cerda et al. (2017) evaluated the total phenolic and flavonoid

Table 2. Toxicity of cannabis extracts on *Myzus persicae*

	% Mortality rates ±SH		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	3.03±0.35 b	7.94±0.20 b	7.94±0.20 b
Maltepe	1.22±0.33 b	6.67±0.55 b	10.17±0.79 b
Narlısaray	4.08±0.48 b	12.02±0.40 ab	19.38±0.11 ab
Kavacık	23.56±0.30 a	26.01±0.21 a	27.27±0.17 a

*Means in a column followed by a different lowercase letter represents results are significantly different (ANOVA $p < 0.05$, Tukey test).

Table 3. Toxicity of cannabis extracts on *Aphis fabae*

	% Mortality rates ±SH		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	5.44±0.44 c	5.44±0.44 c	5.44±0.44 c
Maltepe	50.15±0.19 b	63.29±0.14 b	71.39±0.11 b
Narlısaray	65.76±0.60 b	75.48±0.43 b	77.67±0.46 b
Kavacık	84.33±0.35 a	93.13±0.40 a	96.55±0.44 a

*Means in a column followed by a dizfferent lowercase letter represents results are significantly different (ANOVA $p < 0.05$, Tukey test).

Table 4. Results of dose-death trial of *Cannabis sativa*-Kavacık genotype on *Aphis fabae*

	LD_{50} (mg/individual) (confidence interval)	**LD_{90}(mg/individual) (confidence interval)**
24th hour	0.40 (0.33-0.46)	0.159 (0.122-0.246)
48th hour	0.33 (0.27-0.38)	0.110 (0.91-0.246)

contents of the 10 different genotypes of *Ugni molinae* and stated that the different genotypes had different rates of phenolic and flavonoid compounds. According to Pavel et al. (2014), the obtained essential oil contents and their amounts from different *Mentha* genotypes were different. Moreover, they tested the effectiveness of essential oils obtained from different genotypes of *Mentha* on *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) larvae, and while some genotypes caused high mortality in *C. quinquefasciatus* larvae, others caused lower mortality rates. They stated that this difference was due to the different chemical contents and amounts of them in each genotype.

The sensitivity of some insect species to different plant substances could be variable. Similarly, the different mortality rates observed at aphid species following application of plant extracts derived from different genotypes of cannabis could be attributed to the different compounds and their rates in the plants. Alghamdi (2018) tested the efficacy of *Moringa oleifera* and *Eruca sativa* on *Macrosiphum rosae* and *A. fabae*. While *Moringa oleifera* caused 63.5% mortality, *M. rosae* caused 72.5% mortality in *A. fabae*. *Eruca sativa* caused 97.5% mortality in *M. rosae* and 92.4% mortality in *A. fabae*. Salari et al. (2010) tested the efficacy of *Otostegia persica* extract on three different aphid species and a warehouse insect pest, and after 72 hours, percentages of mortality rates were found to be 57.9% on *M. persicae*, 70.8% on *A. fabae*, 89.5% on *A. gossypii,* and 34.4% on *Trilobium castaneum,* respectively.

There are also many different studies associated with the effectiveness of extracts from different plants against these pest species. For instance, Czerniewicz et al. (2018) tested the toxic effects of plant essential oils obtained from *Santolina chamaecyparissus*, *Achillea millefolium*, *Tanacetum vulgare*, *Tagetes patula,* and *Artemisia absinthium* on *M. persicae* and found LC_{50} values of 0.34%, 0.34%, 0.47%, 0.61% and 0.69%, respectively, after 24 h. The ethanol extract of *Nerium oleander* with a 10% concentration showed 70% mortality on *M. persicae* nymphs (Nia et al., 2018). Mohmed (2019) indicated that *Carissa macrocarpa* extract, caused the toxic effect at 46.6% on *A. fabae*.

CONCLUSION

When the literature was examined, no study was found on the insecticidal activity of cannabis extract on *D. noxia*, *M. persicae,* and *A. fabae*; thus, this research represents the first study on this plant with these pests. It has been aimed at obtaining information about the possibilities of using some industrial cannabis genotypes, whose importance has increased day by day in recent years, especially in the biological control of agricultural pests. It is thought that the chemical differences between these genotypes should be revealed, and their effectiveness against pests should be determined in future studies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the

authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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